Determination of the Contents of A- and B-Starches in Barley using Low Angle Laser Light Scattering

Ivan BOHAČENKO[1], Josef CHMELÍK[2] and Vratislav PSOTA[3]

[1]Food Research Institute Prague, Prague, Czech Republic; [2]Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Brno, Czech Republic; [3]Research Institute of Brewing and Malting, Brno, Czech Republic

Abstract

Bohačenko I., Chmelík J., Psota V. (2006): **Determination of the contents of A- and B-starches in barley using Low Angle Laser Light Scattering**. Czech J. Food Sci., **24**: 11–18.

A method was proposed for the determination of the contents of A- and B-starches in barley and for the use in plant breeding stations and brewing/malting laboratories. A combination was used of classical methods (incl. crushing of barley kernels by a roll crusher, steeping in 0.02M HCl, repeated rubbing and filtering through the sieve of 0.08 mm), and novel pieces of know-how (treatment with β-glucanase and cellulase, alkalisation at pH = 10.0 and centrifugation of crude starch suspension through the layer of CsCl). In this way, barley starch of high purity was obtained at a very low loss, using Low Angle Laser Light Scattering for the determination of the size distribution of starch granules. The boundary of the particle size between the peaks of A-starch and B-starch (7 μm) was evaluated from the distribution curves, while the contents of A- and B-starches were calculated from the cumulative curves. As a whole, the method was internally validated and for its repeatability and uncertainty of measurement the stimates of standard deviation s = 1.56 and confidence interval $L_{1,2} = x \pm 1.9\%$ were established, respectively. Taking into account the generally well known difficulties associated with the extraction of substances from biological materials, in this case the separation of starch from barley kernels, we believe that the proposed method will bring satisfactory results in practice.

Keywords: barley; A-starch; B-starch; starch granules; Low Angle Laser Light Scattering

Based on the long term research in the field of grain starches, it is now generally accepted that the size distribution of starch granules in barley, as well as in wheat, is of bimodal character. The granules of the size in excess of about 10 μm are designated as "A-starch", while the smaller ones are referred as "B-starch" (Lindeboom *et al*. 2004). The resulting average size and size distribution of starch granules are not only specific for individual cereal varieties but are also dependent on the effects of the environment and the agricultural technology acting on the plant in the course of its growth (Davis *et al*. 2003; Palmer 1993; Tester 1997; MacLeod & Duffs 1988; Psota *et al*. 2002).

In the case of barley, the size distribution of starch granules plays the most important role in the production of beer due to its influence on the quality of malt. It was shown that B-starch, relatively firmly incorporated in the lipide-protein matrix (Kang *et al*. 1985), with a higher temperature of gelatinisation as compared to that of A-starch (MacGregor 1991), was less liable to

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QD 1005.

enzymatic attack in the course of malting (Brennan *et al*. 1997). It is presumed that during malting and malt wort preparation only a half of small starch granules is subject to enzymatic degradation, i.e. about 5% of the total starch content will not be fermented into alcohol. Further, residual non-hydrolysed B-starch can cause turbidity and problems in filtration in malt wort production (Tillet & Bryce 1993).

In the course of their development, the methods employed for determining the size distribution of starch granules have seen considerable changes and modifications due to both the recent development of chemical, biological and physical knowledge of the formation and character of starch linkage to other plant materials, and the advanced instrumentation available for the particle size determination. However, any method may be divided into two successive parts, i.e.:

– separation of starch from plant materials and its purification,
– determination of the size distribution of starch granules.

In general, the first part of the process is considered more problematic. In other words, it is more difficult to obtain pure starch, whose composition will correspond to its composition in the original matrix as best as possible, than to measure the particle size of starch granules by a suitable instrumentation (Lindeboom *et al*. 2004).

McDonald and Stark (1988) give the summary in their paper of the methods employed for the separation and purification of cereal starches, including that of barley, in the period betwen 1940–1980. The procedures described are rather simple and consist of the starch separation from ground plant materials by washing with water and the subsequent removal of impurities using multiple filtration on screens combined with centrifugation.

The method by Morrison *et al*. (1984) was widely applied in the years of 1980's and 1990's. Starch was released from crushed barley kernels by maceration in 0.02M HCl for 12 hours. The mixture was then neutralised and neutral protease was added. Crude starch was obtained by repeated rubbing and filtering the crushed kernels through the sieve of 75 μm. It was further purified by multiple centrifugation (10 000 g) and the so-called "brown layer" containing mainly impurities was removed from the surface of the decanted material after each cycle.

McDonald and Stark (1988) made a critical evaluation of this method and objected to the loss of B-starch caused by the repeated removal of the brown layer. They recommended protease to be added later, to the previously separated brown layer. After the centrifugation of the hydrolysed brown layer at 10 000 g, the residual B-starch obtained should be added back to the main portion of the crude starch. The final refined product was obtained by another multiple centrifuging at 10 000 g and shaking with toluene.

The authors of two papers published in 1990 (South & Morrison 1990; Sulaiman & Morrison 1990) employed centrifugation through a layer of CsCl solution for purifying the suspensions of crude starch separated from wheat or barley. In principle, the suspension of crude starch is layered over 80% water solution of caesium chloride and this two-phase system is centrifuged at 14 000–18 000 g. In this process, starch granules pass through the CsCl layer to accumulate at the tube bottom, while the impurities are retained in the CsCl layer. Indisputable advantages of this method consist of a considerable time saving and the reduction of the losses during crude starch refining. You and Izydorczyk (2002) successfully employed the method in the study of barley starches with variable amylase contents. The same authors applied the addition of protease and saccharolytic enzymes lichenase and xylanase to facilitate the release of starch from crushed barley kernels. Similarly, Zheng and Bhatty (1998) obtained improved yields of starch separated from hull-less barley employing the addition of a commercial enzyme mixture containing cellulase, β-glucanase and xylanase, the main effect being ascribed to β-glucanase.

Takeda *et al*. (1999) published a process for the separation of starch from barley in an alkaline medium. The procedure consists of steeping barley kernels in 0.2% NaOH solution for 2 days at the temperature of 4°C, followed by homogenisation and stepwise filtration using a set of sieves of 100, 200 and 400 mesh. Starch obtained in this way was washed with 0.2% NaOH using multiple centrifugation until the disapperance of biuret reaction, and then with water. The method was successfully used in characterising the size of starch granules obtained from quinoa, barley and adzuki seeds (Tang *et al*. 2002).

There are more methods for determining the size of solid particles which are based on various

experimental techniques and it should be noted that almost all such methods have been applied to the determination of the size of starch granules in plant materials. Lindeboom *et al*. (2004) evaluated their advantages and deficiencies in a critical review.

As to barley starches, older procedures employing light microscopy, liquid sedimentation balance (Novotný 1970; Banks *et al*. 1973), and micro-sieving (Baghate & Palmer 1972; Evers *et al*. 1974) were used. Later, in 90's, so-called "Coulter Counter method" was very popular, in which starch granules were classified by size related to their effect on the electrical resistance of an electrically conductive liquid (McDonald & Stark 1988; South & Morrison 1990). Among other microscopic methods in current use is the image analysis of optical microscopy (IAOM) in which the size of starch granules is determined after staining with iodine solution (Baum & Bailey 1987; Tillet & Bryce 1993; You & Izydorczyk 2002), and also the scanning electron microscopy (SEM) should be mentioned. The latter is used particularly for examining, in detail, the morphological characteristics of starches of various plants (Jane *et al*. 1994) and it also finds an application in the verification of the starch granule size in the course of starch fractionation.

Finally, one should mention two novel advanced methods that are gradually more and more used for determining the size of starch granules. The methods concerned comprise the LALLS (Low Angle Laser Light Scattering) and the implementation of FFF (Field Flow Fractionation) techniques in the gravitation or centrifugal field. The two methods are non-destructive, not time-consuming, and are highly objective, as the measured sets contain more than 100 000 particles. In the case of LALLS, the particle size is measured directly, not distinguishing between starch granules and impurities. Therefore, the success of the analysis depends on the use of samples of high purity. The FFF technique may be used for both the size determination and the fractionation of starch granules. As the determination of size by the latter method requires a consistent specific weight of the particles measured, high purity of samples is also essential for the FFF method.

Psota *et al*. (2000) used the LALLS method to distinguish between the contents of A- and B-starch fractions on two varieties of malting barley. Tang *et al*. (2002) used the same method to characterise barley starch and starches of quinoa and adzuki seeds. Moon and Giddings (1993) published the first paper dealing with the separation and size distribution of starch granules using the Sd/St FFF (Sedimentation/Steric Field-Flow Fractionation) method. Contado *et al*. 1999) employed the technique of SPLITT cell (split flow-thin) for starch fractionation. Contado and Dondi (2001) described the combination of SPLITT cell and Sd/St FFF techniques applicable to the determination of the size distribution of starch particles. Chmelík *et al*. (2001) compared the size characteristics of the starch granules of two barley varieties, obtained by the methods of SEM, IAOM, LALLS and GFFF (Gravitational Field-Flow Fractionation). Their evaluation did not differ, in practice, from the evaluation made by Lindeboom *et al*. (2004). Interesting was the fact that the ratio of two main peak areas determined by GFFF corresponded to the mass ratio of A- and B-starch granules determined by LALLS. This finding was supported by the determination of A- and B-starches contents in 10 samples of spring malting barley, as the two methods (GFFF and LALLS) put the samples to the same order according to this parameter (Psota *et al*. 2002).

This contribution extends the first results obtained by Psota *et al*. (2000) where the suitability of the LALLS instrumental method was verified for the determination of the particle size distribution of barley starch granules. However, it should be noted that the procedure employed there for the separation of starch from barley, using washing of cracked, mechanically de-husked kernels with water on a sieve further followed by simple purification of the crude starch obtained by alkalisation to pH 10–11, would be the source of errors increasing the repeatability and uncertainty of the results below the acceptable limit. Therefore, the main objective of our efforts was to offer a reasonably efficient and standardised procedure for the separation and purification of starch from barley kernels which would employ both the classical and the current advanced knowledge. In conclusion, the method as a whole (including the determination of the size distribution by LALLS), was internally validated, i.e. its repeatability and uncertainty of measurement determined.

MATERIALS AND METHODS

Apparatus. pH-meter – Inolab (WTW GmbH, Austria); Shaking water bath – GFL 1083 (Burkmetal GmbH, Germany); Sieve (stainless), diameter

200 mm, mesh size 0.08 mm (Retsch, Germany); Centrifuge – Janetzki K70D (swinging rotor, 4 centrifuging vessels of 1 l volume each, 3000 rpm max.); High speed cooled centrifuge – Model KR25i, 50 000 g max. (JOUAN, France); Laboratory roll crusher (gap width 0.3 mm); Particle Size Analyser – Analysette 22 (Fritsch, Germany); Polarimeter – Sucromat VIS/NIR, (Kernelon, Germany); Ultrasonic bath (Bandeline Electronic, Germany).

Chemicals and enzymatic preparations. Caesium chloride, CsCl (Sigma); enzymatic preparations – β-glucanase 750L, Cellulase 200 L (supplied by Rhodia); other chemicals (NaOH, HCl, etc.) of analytical purity.

Samples. A sample of malting barley of Jersey variety was supplied by the Research Institute of Brewing and Malting, Czech Republic, Brno.

Determination of starch content in barley and in residue of washed crushed barley kernels. Starch contents were determined by the polarimetric method of Ewers (Davídek 1977) using the conversion factor of 1.912 for barley starch, and were converted to the dry matter of the two materials.

Determination of starch granule size distribution

Separation and purification of starch from barley kernels. Whole barley kernels (15 g, approx.) were cracked by passing them through the laboratory roll crusher two times. The cracked grains were then steeped in 100 ml 0.02M HCl at 4°C for 15 h (usually overnight). After warming the sample to the room temperature, its pH was adjusted to 5.0 ± 0.2 with 0.2M NaOH. Solutions of cellulase (0.5 ml) and β-glucanase (0.5 ml) were added. A flask with the mixture was then placed in a shaking water bath of the temperature of 30°C. Then, the mixture was stirred intensively several times in the course of shaking. After removing the sample from the bath, its pH was adjusted to 10.0 ± 0.2 with 0.2M NaOH and the flask was placed again in the shaking water bath whose temperature was increased to 50°C for 1.5 h. Then the mixture was left to cool to room temperature, its pH was adjusted to 7.0 ± 0.2 using 0.2M HCl, and it was filtered through a sieve of 0.08 mm mesh size. The filtrate containing starch was placed in a storage vessel. The digested material retained on the sieve was mixed with water (100 ml approx.) and gently rubbed using a mortar and a pestle to release the remaining starch granules. It was then filtered and the filtrate with the starch was added to the storage vessel. The procedure was repeated three more times. The residue of crushed kernels was washed with 50 ml of water and dried. The combined filtrates (approx. 600 ml) were centrifuged at 3000 rpm for 30 min. After removing the liquid fraction, the solid starch fraction retained in the centrifuging vessel was stirred up in water (500 ml) and centrifuged once again.

Crude starch obtained in this way was transferred into a 100 ml ground-neck flask and homogenised in an ultrasonic bath for 15 min. Three millilitres of the homogenised crude starch suspension were carefully layered over 4 ml of 80% water solution of CsCl in centrifuge tubes. After centrifugation (14 000 g/30 min), the upper layer (consisting of water phase and CsCl solution with impurities) was carefully removed. The remaining solid starch was washed two times using the addition of 7 ml of distilled water and centrifugation (14 000 g for 30 min). Finally, the refined starch was transferred with 5 ml of distilled water into a vial. A drop of formaldehyde was added and the product was stored at 4°C in a refrigerator.

Size distribution of starch granules. The measurement of the size distribution of starch granules was carried out using the Particle Sizer Analyser Analysette 22. Starch was dosed in the instrument in the form of dense suspension. The data necessary for calculating the size distribution of starch granules were obtained from the distribution and the cumulative curves. The distribution curves served for the evaluation of granule size boundary between the peaks of A- and B-starches. The cumulative curves (or their tabular values) describe the relationship between the volume proportion of a given starch fraction and the size of granules in that fraction. Provided that all the starch granules have the same specific weight, the given per cent by volume may be well considered as per cent by weight.

Measurement conditions

Measuring range	0.18–300.74 μm
Resolution	124 channels (10 mm/114 mm)
Absorption	13.00%
Measurement duration	20 scans
Pump	167 rpm
Stirrer	153 rpm

Repeatability and uncertainty of measurement (Eckschlager *et al*. 1980).

Repeatability of the determination of A- and B-starch fractions was expressed as the estimate of standard deviation and calculated using the following formula:

$$s = k_n \times R$$

where:
s – estimate of standard deviation
n – number of parallel determinations
k_n – tabular value
R – range of results (difference between the highest and the lowest results)

Uncertainty of determination of A- and B-starch fractions was expressed as the width of the interval of confidence, calculated by the following formula:

$$L_{1,2} = x \pm K_n \times R$$

where:
$L_{1,2}$ – upper and lower limits of the confidence interval
x – average value of all the determinations
n – number of parallel determinations
K_n – tabulated coefficient of n parallel determinations at a chosen level (1-α)
R – range of results (difference between the highest and the lowest result)

DISCUSSION

As mentioned in the introduction, the main objective of this work was to propose a method for the determination of A-and B-starches in barley, based on the starch granule size distribution. As it was intended for the evaluation of this parameter in plant breeding stations and brewing/malting laboratories, it should be sufficiently representative and reproducible and, at the same time, as quick as possible and eligible for standardisation.

A sample weighing 15 g was used for one analysis, i.e. the quantity that, in our opinion provided a sufficiently representative result. However, the weight may be increased if required. The flow diagram of the proposed procedure of the starch separation and refining is shown in Figure 1.

From this diagram it follows that the operations of whole barley crushing, steeping in HCl, and crude starch separation were made according to the procedure of McDonald and Stark (1988). The improved release of starch from the crushed kernels was accomplished in two stages. First, the addition of two saccharolytic enzymes, cellulase and β-glucanase, was employed. Xylanase recommended by You and Izydorczyk (2002) was not used, as our preliminary tests showed that its addition did not significantly improve

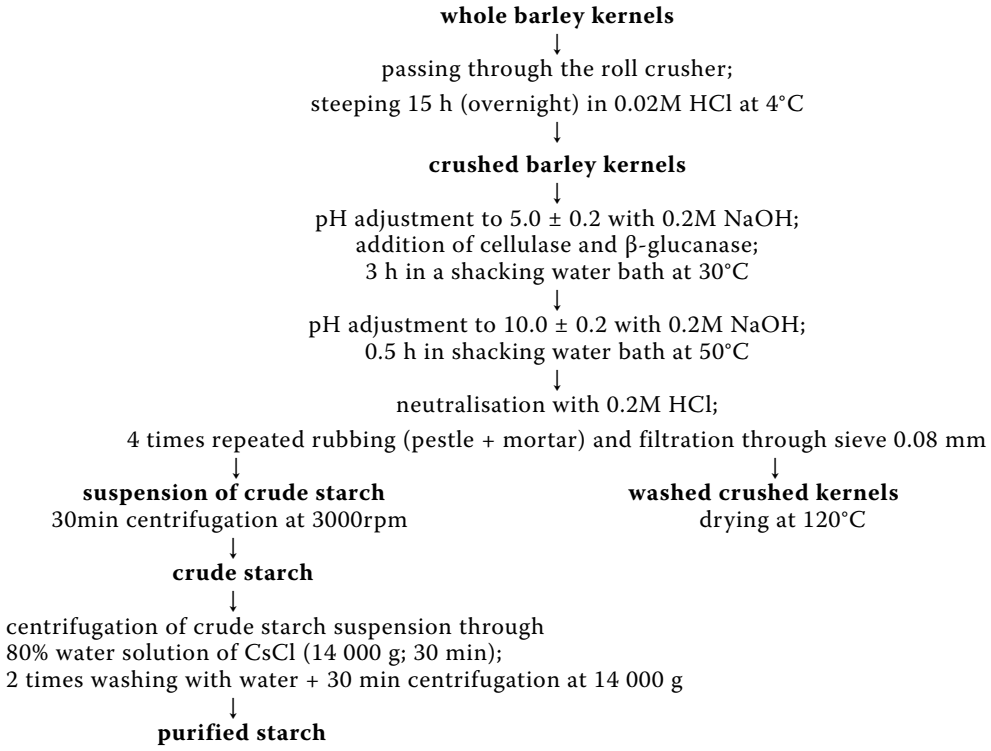

Figure 1. Procedure of starch separation and purification

the effect of the other two enzymes on the starch release. In the second phase, the time consuming and experimentally demanding treatment with protease after Morrison *et al.* (1984) (digestion at 4°C overnight under continuous stirring) was replaced with the treatment in alkaline medium (pH 10, approx.) at 50°C for 1.5 h.

Starch extraction efficiency was assessed using the mass balance of the starch contents in barley kernels and in washed crushed kernels. It was proved that about 7.5% of the initial quantity of starch was retained in the washed crushed kernels, i.e. that the yield of about 90–93% of crude starch was obtained in the process of separation. This result was comparable with the data given by Morrison *et al.* (1984).

Crude starch purification was made by the method of South and Morrisson (1990) using centrifugation of starch suspension through a layer of CsCl solution. This step allowed us to reduce the time of analysis considerably as compared to the procedure of McDonald and Stark (1988) consisting of the release of B-starch from the brown layer by the action of protease and final starch refining by shaking with toluene. The proper size distribution of starch granules itself was determined by LALLS in purified starch.

Considering the proposed practical use of the method of concern, its internal validation was made at the level of determination of A- and B-starches in barley. The entire procedure was taken into account, i.e. the starch separation and refining, including the determination of the starch granule size distribution by LALLS. We performed the validation by determining an estimate of standard deviation and confidence interval in the analysis of the same sample of barley repeated five times. The granule size of 7 μm was chosen as the boundary value between A- and B-starches based on the course of the distribution curves (Figure 2). The contents of A- and B-starches were calculated in the individual determinations from the cumulative curves (Figure 2) and are shown in Table 1.

Using the tabular values of coefficients k_n = 0.4299 and K_n = 0.51 (at the level of (1-α) = 0.95), the standard deviation was estimated at s = 1.56 and the confidence interval $L_{1,2} = x \pm 1.9\%$ was calculated. As the sum of A- and B-starches volumes is normalised to 100% in any cumulative curves, the validation values apply equally to the two starch fractions.

Within the frame of a previous work (Příhoda *et al.* 1999), the validation was made of the proper determination of the starch granule size distribution using Analysette 22. The determination was made in nine times repeated measuring of 10% aqueous suspension of refined wheat starch dosed directly in the instrument measuring cell. The estimates of the standard deviation s = 0.24 and confidence interval $L_{1,2} = x \pm 0.16\%$ were calculated for B-starch. It can be reasonably expected that similar values would be obtained for barley starch, too. As the values are relatively very low, the fluctuation of results should be assigned to the process of preparation of pure starch samples from barley. Particularly, the repeated rubbing and filtering through the sieve in the preparation of crude starch suspension is the critical step that can be standardised in practice only with difficulties. More attention should be paid also to the starch

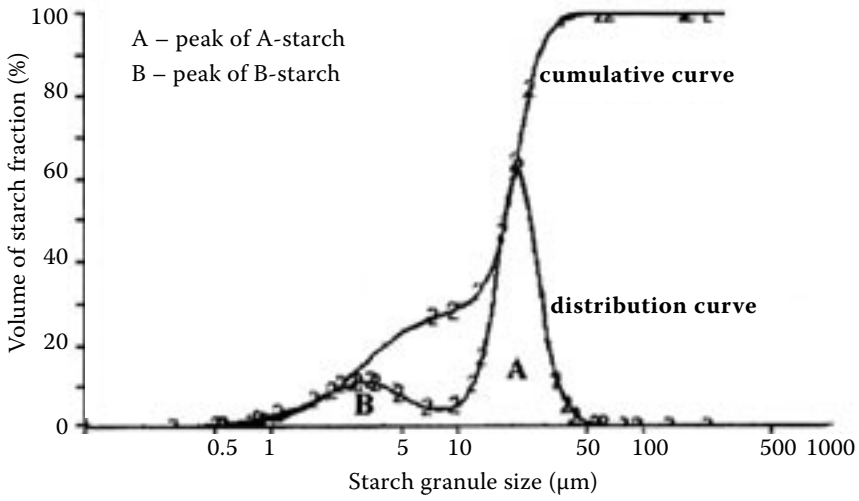

Figure 2. Distribution and cumulative curves of barley starch granule sizes

Table 1. Results of five times repeated determination of A- and B-starches in barely

Analyse No.	Content (%)	
	B-starch	A-starch
1	27.03	72.97
2	25.57	74.43
3	23.48	76.52
4	23.39	76.61
5	25.19	74.81
Ø	24.93	75.07

suspension handling as the suspensions could readily sediment (losing their homogenity) and adversely affect the results of the particle size distribution analysis.

However, we were not able to compare our results of validation with other authors' data as the latter were not available in the literature cited. Nevertheless, taking into account the generally well known difficulties associated with the extraction of substances from biological materials, including the separation of starch from barley kernels, we believe that the method proposed will bring satisfactory results and find good use in practice. We would like to note that this method can also be used in other applications of the ize determination of barley starch kernels.

References

Baghate G.N., Palmer G.H. (1972): A reassessement of the chemical structure of barley and wheat starch granules. Die Stärke, **24**: 336–341.

Banks W., Greenwood C.T., Muir D.D. (1973): Studies on the biosynthesis of starch granules. Die Stärke, **25**: 225–230.

Baum B.R., Bailey L.G. (1987): A survey of endosperm starch granules in the genus *Hordeum*: a study using image analytic and numerical taxonomic techniques. Canadian Journal of Botany, **65**: 1563–1569.

Brennan C.S., Amor M.A., Harris N., Smith I., Cantrell I., Griggs D., Shevry P.R. (1997): Cultivar differences in modification patterns of protein and carbohydrate reserves during maltin of barley. Journal of Cereal Science, **26**: 83.

Contado C., Dondi F. (2001): Barley starch granules subject to SPLITT cell fractionation and Sd/Stfff size characterization. Starch/Stärke, **53**: 414–423.

Contado C., Riello F., Blo G., Dondi F. (1999): Continuous split flow-thin cell fractionation of starch particles. Journal of Chromatography A, **845**: 303–316.

Davídek J. (1977): Laboratorní příručka analýzy potravin. SNTL, Praha: 254–255.

Davis J.P., Supatcharee N., Khandelwal R.L., Chibbar R.N. (2003): Synthesis of Novel starches in planta: opportunities and challenges. Starch/Stärke, **55:** 107–120.

Eckschlager K., Horsák I., Kodejš Z. (1980): Vyhodnocování analytických výsledků a metod. SNTL, Praha.

Evers A.D., Greenwood C.T., Muir D.D., Venables C. (1974): Studies on the biosynthesis of starch granules. Die Stärke, **26**: 42–46.

Chmelík J., Krumlová A., Budinská M., Kruml T., Psota V., Bohačenko I., Mazal P., Vydrová H. (2001): Comparison of size characterization of barley starch granules determined by electron and optical microscopy, Low Angle Laser light scattering and gravitational field-flow fractionation. Journal of the Institute of Brewing, **107**: 11–17.

Jane J.-L. , Kasemsuwan T., Leas S., Zobel H., Robyt J.F. (1994): Anthology of starch granule morphology by scanning electron microscopy. Starch/Stärke, **46**: 121–129.

Kang M.Y., Sugimoto Y., Kato I., Sakamoto S., Fuwa H. (1985): Some properties of large and small starch granules of barley endosperm. Agricultural and Biological Chemistry, **49**: 1291–1297.

Lindeboom N., Chang P.R., Tyler R.P. (2004): Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch/Stärke, **56**: 89–99.

MacGregor A.W. (1991)**:** In: Proceedings of the European Brewery Convention Congress, Lisabon: 37.

MacLeod L.C., Duffs C.M. (1988): Temperature effects on starch granules in developing barley starch. Journal of Cereal Science, **8**: 29–37.

McDonald A.M.L., Stark J.R. (1988): A critical examination of procedures for the isolation of barley starch. Journal of the Institute of Brewing, **94**: 125–132.

Moon M.H., Giddings J.C. (1993): Rapid separation and measurement of particle size distribution of starch granules by sedimentation/steric field-flow fractionation. Journal of Food Science, **58**: 1166–1171.

Morrison W.R., Milligan T.P., Azudin M.N. (1984): A relationship between the amylose and lipid contents of starches from diploid cereals. Journal of Cereal Science, **2**: 257–251.

Novotný F. (1970): Příspěvek ke zjištění kvality škrobu u sladovnických ječmenů. Rostlinná výroba, **16**: 1105–1118.

Palmer G.H. (1993): Ultrastucture of endosperm and quality. Fermentation, **6**: 105.

Příhoda J., Bohačenko I., Vydrová H., Svobodová A. (1999): Technologická kvalita pšeničných mouk jako jeden z rozhodujících faktorů rozvoje a modernizace výroby pšeničného škrobu v ČR. [Výroční zpráva projektu č. EP 7203 za rok 1998.] Praha.

Psota V., Bohačenko I., Pytela J., Vydrová H., Chmelík J. (2000): Determination of size distribution of barley starch granules using low angle laser light scattering. Rostlinná výroba, **46**: 443–436.

Psota V., Bohačenko I., Hartmann J., Budínská M., Chmelík J. (2002): Comparison of the GFF and LALLS methods for the measurement of starch granule size distribution in spring barley caryopses. Journal of the Institute of Brewing, **108**: 200–203.

South J.B., Morrison W.R. (1990): Isolation and analysis of starch from single kernels of wheat and barley. Journal of Cereal Science, **12**: 43–51.

Sulaiman B.D., Morrison W.R. (1990): Proteins associated with the surface of wheat starch granules purified by centrifuging through caesium chloride. Journal of Cereal Science, **12**: 53–61.

Takeda Y., Takeda Ch., Mizukami H., Hanashiro I. (1999): Structures of large, medium and small starch granules of barley grain. Carbohydrate Polymers, **38**: 109–114.

Tang H., Watanabe K., Mitsunaga T. (2002): Characterization of storage starches from quinoa, barley and adzuki seeds. Carbohydrate Polymers, **49**: 13–22.

Tester R.F. (1997): Influence of growth condition on barley starch propereties. International Journal of Biological Macromolecules, **21**: 37–45.

Tillet I.J.L., Bryce J.H. (1993): The regulation of starch granule size in endosperm of developing barley grains. In: Proceeding of the 24th European Brewery Convention Congress, Oslo: 45–52.

Zheng G.H., Bhatty R.S. (1998): Enzyme-assisted wet separation of starch from other seed components of hull-less barley. Cereal Chemistry, **75**: 247–250.

You Sang Guan, Izydorczyk M.S.(2002): Molecular characteristics of barley starches with variable amylose content. Carbohydrate Polymers, **49**: 33–42.

Received for publication July 1, 2005
Accepted August 9, 2005

Corresponding author:

Ing. Ivan Bohačenko, CSc., Výzkumný ústav potravinářský Praha, Radiová 7, 102 31 Praha 10-Hostivař, Česká republika
tel.: + 420 296 792 379, fax: + 420 272 701 983, e-mail: i.bohacenko@vupp.cz